The assays utilizing nanoparticle-labeled probes were significantly more sensitive than those utilizing fluorophore-labeled probes. Hybridization signal could be resolved at the N=A elements at target concentrations as low as 50 fM (or, for a hybridization chamber containing 20 μL of solution, 1 x $10^6$ total copies); this represents a dramatic increase in sensitivity over common Cy3/Cy5 fluorophore-labeled arrays, for which ~ 1 pM or greater target concentrations are typically required. The higher melting temperatures observed for nanoparticle-target complexes immobilized on surfaces undoubtedly contribute to array sensitivity. The greater stability of the probe/target/surface-oligonucleotide complex in the case of the nanoparticle system as compared with the fluorophore system presumably results in less target and probe lost during washing steps.

Colorimetric, nanoparticle labeling of combinatorial oligonucleotide arrays will be useful in applications such as single nucleotide polymorphism analysis, where single mismatch resolution, sensitivity, cost and ease of use are important factors. Moreover, the sensitivity of this system, which has yet to be totally optimized, points toward a potential method for detecting oligonucleotide targets without the need for target amplification schemes such as polymerase chain reaction.

Example 20: Nanoparticle Structures

The reversible assembly of supramolecular layered gold nanoparticle structures onto glass supports, mediated by hybridized DNA linkers, is described. Layers of oligonucleotide-functionalized nanoparticles were successively attached to oligonucleotide-functionalized glass substrates in the presence of a complementary DNA linker. The unique recognition properties of DNA allow the nanoparticle structures to be assembled selectively in the presence of the complementary linker. In addition, the structures can be assembled and disassembled in response to external stimuli which mediate hybridization of the linking duplex DNA, including solution temperature, pH, and ionic strength. In addition to offering a very selective and controlled way of building nanoparticle based architectures on a solid

support, this system allows one to study the factors that influence both the optical and melting properties of nanoparticle network structures linked with DNA.

Others have demonstrated how bifunctional organic molecules (Gittins et al., *Adv. Mater.* **11**:737 (1999); Brust et al., *Langmuir* **14**:5425 (1998); Bright et al., *Langmuir* **14**:5695 (1998); Grabar et al., *J. Am. Chem. Soc.* **118**:1148 (1996); Freeman et al., *Science* **267**:1629 (1995); Schmid et al., *Angew. Chem. Int. Ed. Engl.* **39**:181 (2000); Marinakos et al., *Chem. Mater.* **10**:1214 (1998)) or polyelectrolytes (Storhoff et al., *J. Am. Chem. Soc.* **120**:1959 (1998); Storhoff et al., *J. Cluster Sci.* **8**:179 (1997); Elghanian et al., *Science* **277**:1078 (1997); Mirkin et al., *Nature* **382**:607 (1996)) can be used to controllably construct mono- and multilayered nanoparticle materials off of planar substrates. The attractive feature of using DNA as a nanoparticle interconnect is that one can synthetically program interparticle distances, particle periodicities, and particle compositions through choice of DNA sequence. Moreover, one can utilize the reversible binding properties of oligonucleotides to ensure the formation of thermodyanamic rather than kinetic structures. In addition to providing a new and powerful method for controlling the growth of nanoparticle-based architectures from solid substrates, this strategy also allows one to evaluate the relationship between nanoparticle aggregate size and both melting and optical properties of aggregate DNA-interlinked structures. An understanding of these two physical parameters and their relationship to materials architecture is essential for utilizing nanoparticle network materials, especially in the area of biodetection.

The oligonucleotide-functionalized, 13-nm-diameter gold nanoparticles used to construct the multilayer assemblies were prepared as described in Examples 1 and 3. The nanoparticles had 5'-hexanethiol-capped oligonucleotide **1** (5'-$HS(CH_2)_6O(PO_2^-)$O-CGCATTCAGGAT-3' [SEQ ID NO:50]) and 3'-propanethiol-capped oligonucleotide **2** (3'-$HS(CH_2)_3O(PO_2^-)$O-ATGCTCAACTCT-5' [SEQ ID NO:59]) attached to them to yield nanoparticles ***a*** and ***b***, respectively (see Figure 37). Glass slides were functionalized with 12-mer oligonucleotide **2** as described in Example 10. To build nanoparticle layers, the substrates were first immersed in a 10 nM solution of 24-mer linker **3** (5'-

TACGAGTTGAGAATCCTGAATGCG-3' [SEQ ID NO:60]) and allowed to hybridize with it for 4 hours at room temperature (see Figure 37). The substrates were washed with clean buffer solution, and then hybridized with a 2 nM solution of particle ***a*** for 4 hours at room temperature to attach the first nanoparticle layer. A second nanoparticle layer could be attached to the first one by similarly exposing the surface to solutions of linker **3** and nanoparticle ***b***. These hybridization steps could be repeated to attach multiple, alternating layers of nanoparticles ***a*** and ***b***, each layer connected to the previous one by linker **3**. In the absence of linker, or in the presence of noncomplementary oligonucleotide, no hybridization of nanoparticles to the surface was observed. In addition, multilayer assembly was only observed under conditions which promoted the hybridization of the DNA linkers: neutral pH, moderate salt concentration (> 0.05 M NaCl), and a temperature below the duplex melting temperature ($T_m$).

Each hybridized nanoparticle layer imparted a deeper red color to the substrate, and after ten hybridized layers, the supporting glass slide appeared reflective and gold in color. Transmission UV-vis spectroscopy of the substrate was used to monitor the successive hybridization of nanoparticle layers to the surface, Figure 38A. The low absorbance of the initial nanoparticle layer suggests that it seeded the formation of further layers, which showed a near linear increase in the intensity of the plasmon band with each additional layer (for each successive nanoparticle layer formation, no additional absorbance was observed on exposure for longer times or to higher concentrations of either linker **3** or nanoparticle solution). The linearity of the absorbance increase after the generation of the initial nanoparticle layer indicates that the surface was saturated with hybridized nanoparticles with each successive application, Figure 38B. This is supported by field-emission scanning electron microscope (FE-SEM) images of one (Figure 39A) and two (Figure 39B) nanoparticle layers on a surface, which show low nanoparticle coverage with one layer, but near complete coverage with two layers. The $\lambda_{max}$ of the plasmon band for the multilayer assemblies shifts no more than 10 nm, even after 5 layers. The *direction* of this shift is consistent with other experimental (Grabar et al., *J. Am. Chem. Soc.* **118**:1148 (1996)) and